EPA Reviewer: Kit Farwell, DVM Signature: _____

Reregistration Branch I, Health Effects Division (7509C) Date_____

EPA Branch Senior Scientist: Whang Phang, PhD Signature: _____

Reregistration Branch I, Health Effects Division (7509C) Date_____

TXR#: 0051063

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat; OPPTS 870.7485 [§85-1)]; OECD 417.

DP BARCODE: D291213
P.C. CODE: 030801 (2,4-DB) and 030819 (2,4-DB-DMA)

TEST MATERIAL: 2,4-DB (99% radiochemical purity)
2,4-DB-DMA (99% radiochemical purity)

SYNONYMS: 4-(2,4-dichlorophenoxy)butyric acid

CITATIONS: Kraut, G.R., Gibson, N.A. and Marsh, J.D. (1995) The Disposition and Metabolism of [^{14}C]2,4-DB Dimethylamine Salt in the Rat. PTRL East, Inc., Richmond, KY. Laboratory Report No.1840, October 13, 1995. MRID 43830101. Unpublished.

SPONSOR: 2,4-DB Task Force, 5116 Wood Valley Drive, Raleigh, NC

EXECUTIVE SUMMARY: In this rat metabolism study (MRID 43830101), two adult CD (Crl:CD® (SB)BR) rats/sex/group were administered a single oral (gavage) dose of [^{14}C]2,4-DB acid or [^{14}C]2,4-DB DMA at target doses of 5.0 mg/kg or 6.0 mg/kg, respectively. Actual doses were 5.3 and 6.7 mg/kg. Three rats/sex/group served as a vehicle control (2% aqueous methylcellulose).

With both treated groups, the majority of the radioactivity (96-97% of the dose) was excreted in the urine, most within 24 hours post-treatment. Fecal excretion accounted for ≤6% of the dose. Levels of expired $^{14}CO_2$ accounted for ≤0.1% of the dose. Material balances between treated groups were comparable, accounting for 102-104% of the dose.

HPLC analysis of whole urine showed that ten metabolites were chromatographically resolved. Of these, five metabolites accounted for ≥5% of the dose. The major urinary metabolite was identified as 2,4-dichlorophenoxy)acetic acid (2,4-D), accounting for 31-44% and 47-53% of the dose in males and females, respectively. Three other major urinary metabolites (U2, U3 and U6) were identified as conjugated forms of 2,4-D, 2,4-dichlorophenol (2,4-DCP) and 2,4-DB, respectively. HPLC analysis of urine from female rats treated with 2,4-DB DMA showed that metabolites U2, U3 and U5/U6 accounted for 9, 23 and 6% of the urinary radiocarbon, respectively. An unknown urinary metabolite (U8), which

accounted for 8-12% of the dose, was identified by mass spectrometry as 4-(2,4-dichlorophenoxy)-3-hydroxybutyric acid (2,4-DB-OH).

HPLC analysis of fecal extracts identified metabolites as 2,4-D, 2,4-DB-OH and 2,4-DCP, accounting for 1.1-2.6, 0.3-0.7 and 0.6-0.9% of the dose, respectively. Trace amounts of unmetabolized 2,4-DB accounted for 0.7-1.1% of the dose.

Based on the analyses, a metabolic pathway was proposed. It appears that 2,4-DB DMA dissociates to the 2,4-DB (free acid) and then shares the same metabolic pathway as the 2,4-DB acid, which includes oxidation, hydrolysis and conjugation.

This metabolism study in the rat is classified acceptable (non-guideline) and does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rat. The study was originally intended as a pilot metabolism study and as such, does not meet the guideline requirements. However, the individual animal data did not show large variations in the results, and the data were informative in demonstrating that the metabolism of 2,4-DB and 2,4-DB DMA is similar.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. No Flagging statement was provided.

I. MATERIALS AND METHODS

A. Materials

1. Test compounds:
   [^{14}C-ring-UL]2,4-DB
   Radiochemical purity: 99%
   Specific activity: 16.12 mCi/mM
   Lot No.: 2792-168

   2,4-DB (unlabeled)
   Purity: ≥98.4% a.i.
   Lot No.: 8812016
   Description: Not specified
   Contaminants: Not specified
   Storage: Not reported
   CAS #: 94-82-6
   Structure:

2. Vehicle: 2% methylcellulose solution

3. Test animals: Species: Rat
   Strain: CD (Crl:CD® (SB)BR)
   Age: adult
   Weight at study initiation: 193-245 g
   Source: Charles River Laboratories, Portage, MI
   Housing: During acclimation, individually housed in polycarbonate shoebox cages. During the in-life phase, animals were housed individually in glass metabolism cages designed for separate collection of urine and feces.
   Acclimation period: at least 7 days
   Diet: Purina Laboratory Rodent Chow 5002 ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 22.5-27.5°C (Appendix 11)
   Humidity: 36-60% (Appendix 11)
   Air Changes: 12-18/hr
   Photoperiod: 12-hr photoperiod

4. Observations: Animals were observed daily for signs of toxicological or pharmacological effects.

5. Preparation of dosing solutions: To prepare [^{14}C]2,4-DB DMA, isotopically diluted [^{14}C]2,4-DB (in solvent) was dried under nitrogen, redissolved in anhydrous methyl alcohol and saturated with dimethylamine. The prepared salt was then dried and redisssolved in an aqueous solution of 2.0 methylcellulose.

   For the [^{14}C]2,4-DB acid preparation, isotopically diluted [^{14}C]2,4-DB (in solvent) was dried under nitrogen and redissolved in absolute ethanol before the addition of an aqueous solution of 2.0% methylcellulose.

   All prepared solutions were refrigerated until used. The solutions were allowed to stand at room temperature for approximately 30 minutes before dosing.

B. Study Design -This study was originally designed as a pilot study; however, based on the results which suggested that the disposition and metabolism of the acid and salt were virtually identical, the sponsor considered the data sufficient to allow EPA to determine that Guideline 85-1 is satisfied.

The study consisted of two groups of Crl:CD:BR rats (2 /sex/dose group) that were dosed once with [^{14}C]2,4-DB acid or [^{14}C]2,4-DB DMA orally (gavage) at target doses of 5.0 mg/kg or 6.0 mg/kg, respectively. Three rats/sex/group served as a vehicle (2% aqueous methylcellulose) The target radioactivity administered for treated animals was 30-50 µCi/rat. Actual doses for each test group are presented in Table 1.

No information was provided regarding dose level selection.

The in-life portions of these studies were conducted from July 14, 1994 to August 12, 1994.

Table 1. Dose groups for [^{14}C]2,4-DB acid or [^{14}C]2,4-DB DMA metabolism study [a].

Dose Group	Target dose (mg/kg)	Actual average ^{14}C-dose (mg/kg)	# animals per group	Target Radioactivity Administered (µCi/rat)	Actual Radioactivity Administered (µCi/rat)
Control	vehicle only	0	3 males 3 females	none	none
2,4-DB	5.0	5.3	2 males 2 females	30-50	41.0
2,4-DB DMA	6.0	6.7	2 males 2 females	30-50	42.2

a Data were obtained from pages 17and 28 of MRID 43830101

1. Dosing - The test animals were dosed orally by gavage with a target dose of 5.0 mg/kg [^{14}C]2,4-DB or 6.0 mg/kg [^{14}C]2,4-DB DMA and a target volume of 0.5-1.0 mL/animal.

Animals were not fasted prior to or after dosing. Animals were weighed prior to ^{14}C-dosing to determine dose per animal and the actual dose administered was determined by weighing the syringe and catheter assembly prior to and immediately after dosing.

2. Sampling - Radiocarbon concentrations in the urine, feces and carbon dioxide trapping solution of the treated animals were quantitated at 0.3, 1 and 2 days post-dosing. Urine and feces samples were stored frozen until time of radioassay. Immediately following the collection of urine and feces, each cage was rinsed with 100 mL of water and the volume of cage wash recorded. After the final collection on Day 3, the cages were rinsed with 25 mL of acetone followed by 75 mL of water. A 15-20 mL subsample of cage wash was stored at room temperature until the radioassay. A carbon dioxide trapping solution (400 mL of 10% sodium hydroxide w/v) was collected and measured. A 15-20 mL subsample was retained at room temperature until the radioassay. Animals were sacrificed three days after treatment and radiocarbon concentrations in the residual carcass and gastrointestinal tract (with contents) were quantitated by combustion analysis.

3. Radioassay - Samples of urine were analyzed for total radioactivity directly by liquid scintillation counting (LSC). Feces and tissue samples were homogenized and radioassayed by LSC following combustion.

4. Metabolite characterization in excreta - Composited whole urine samples from both sexes of treated animals were chromatographically profiled by High Performance Liquid Chromatography (HPLC) System 1. To determine the presence of conjugated metabolites, the urine from female rats treated with [^{14}C]2,4-DB DMA was acid hydrolyzed. Pre- and post-hydrolyzed samples were chromatographically profiled by HPLC System 1. Composited fecal samples from males and females in the treated groups were soxhlet extracted with methanol, concentrated by rotary evaporation and chromatographically profiled by HPLC System 1. Urinary metabolite U8 was the only significant (≥5% of the dose) metabolite that was not identified with reference substances. HPLC System 2 was used to purify metabolite U8 for spectroscopic analyses. Two types of LC/MS analysis, thermospray and electrospray, were used to analyze U8.

5. Statistics - Mean and substance deviations were the only statistical analyses performed.

II. RESULTS

A. Observations - It was stated that no changes in appearance and behavior or overt signs of toxicity were noted in the treated animals during the study.

B. Absorption, excretion, and distribution - The study report states that the urine and feces excretion patterns were found to be virtually identical between male and female rats administered [^{14}C]2,4-DB and [^{14}C]2,4-DB DMA. In both treated groups, 96-97% of the administered radioactivity was recovered in the urine (including cage wash), most within 24 hours of dosing, indicating that both compounds were readily bioavailable and excreted rapidly in the urine. Fecal excretion accounted for < 6% of the dose. Expired CO_2 accounted for ≤0.1% of the dose. Data are presented in Table 2.

Table 2. Percent of administered radioactivity in excreta of rats following a single oral dose of [^{14}C]2,4-DB or [^{14}C]2,4-DB DMA [a]

Dose Group	Sex	Urine and Cage Wash	Feces	Expired Radiocarbon	Total Excreted Radiocarbon	Radiocarbon Remaining in Tissues	Total Recovery
2,4-DB	Male	94.7	6.7	0.0	101.4	1.0	102.4
	Female	96.6	4.3	0.1	101.0	1.0	102.0
	Mean=	95.7	5.5	0.1	101.2	1.0	102.2
2,4-DB DMA	Male	98.1	4.5	0.0	102.6	1.2	103.8
	Female	95.8	5.1	0.0	100.9	1.2	102.1
	Mean=	97.0	4.8	0.0	101.8	1.2	103.0

a Extracted from Table IV, page 40 of MRID 43830101

C. Metabolite Characterization

1. Metabolites in excreta - The study report states that with the HPLC System 1 analysis of whole urine, the metabolite profiles were quantitatively and qualitatively similar between the two treated groups, with some minor quantitative differences between male and female animals (Tables 3 and 4). Ten metabolites were chromatographically resolved in the urine samples. They were named by their HPLC elution order so that the most polar metabolite which was eluted first was U1 and the least polar which eluted last was U10. Five metabolites (U2, U3, U6, U7 and U8) were present in significant concentrations (≥5% of the dose) with both [^{14}C]2,4-DB and [^{14}C]2,4-DB DMA.

   The TLC analysis of extracted urine from rats treated with both [^{14}C]2,4-DB and [^{14}C]2,4-DB DMA also had metabolite profiles that were quantitatively and qualitatively similar, with some minor differences between males and females. Five major bands seen

on the TLC autoradiogram corresponded to the five metabolites during the HPLC analysis.

HPLC System 1 analysis of the fecal samples showed that the metabolite profiles were quantitatively and qualitatively similar between the two treated groups, with some minor quantitative differences between male and female animals. Ten metabolites were also chromatographically resolved in the fecal samples. Since there were relatively low amounts of administered radiocarbon in the feces, only those components that matched available reference substances were further characterized.

Composited urine from female rats treated with [^{14}C]2,4-DB DMA was acid hydrolyzed to determine if conjugated metabolites were present. HPLC System 1analysis of the pre-hydrolyzed sample showed a metabolic profile comparable to its original metabolite profile but with improved separation between metabolites (Table 5). The major metabolite (U7) co-eluted with the 2,4-D reference substance and a trace component (U9) co-eluted with the 2,4-DCP reference substance. After acid analysis, the metabolite profile was changed. The relatively polar urinary components (U2, U3, U5 and U6) in the pre-hydrolyzed sample had almost completely disappeared, which suggested that these components were conjugated metabolites, typically glucuronides and /or sulfates in the rat. In addition, a newly formed trace component (U11) was found to co-elute with the 2,4-D reference. Based on these changes, it was concluded that metabolite U2 is a conjugate of 2,4-D, metabolite U3 is a conjugate of 2,4-DCP and metabolites U5 and U6 are conjugates of U8 and 2,4-DB.

Since urinary metabolite U8 did not co-chromatograph with any of the reference substances, it was isolated and purified for mass spectral analysis using 2,4-DB as a reference compound. Based on positive ion thermospray mass spectrometry, it was concluded that U8 is 4-(2,4-dichloro-phenoxy)-3-hydroxybutyric acid (2,4-DB-OH).

HPLC System 1 analysis of the fecal extracts indicated that the major components were 2,4-D, 2,4-DB-OH, 2,4-DCP and unmetabolized 2,4-DB. Metabolite identification of three of these components were confirmed by TLC System 3 analysis, which showed co-migration of the radioactive components with 2,4-D, 2,4-DCP and 2,4-DB reference substances.

Based on the similarities in absorption, distribution , excretion and metabolism of [^{14}C]2,4-DB DMA and [^{14}C]2,4-DB, it was concluded that the salt dissociates to the free acid in the rat stomach and the two chemicals can be considered equivalent *in vivo*. Both the salt and the acid share the same metabolic pathway in the rat, which includes oxidation, hydrolysis and conjugation.

Table 3: Metabolic Profiles of Whole Urine and Feces Extracts in Rats Treated with [^{14}C]2,4-DB DMA [a]

Sex/Material	Material Balance	U1	2,4-D & 2,4-DCP Conjugates U2/U3	U4	2,4DB-OH Conjugate U5	2,4-DB Conjugate U6	2,4-D U7	2,4-DB-OH U8	2,4-DCP U9	U10	2,4-DB U11
Male Urine	% HPLC % in urine % dose	2.5 98.1 2.5	34.0 98.1 33.4	0.9 98.1 0.9	2.3 98.1 2.3	6.8 98.1 6.7	44.7 98.1 43.9	8.1 98.1 7.9	0.7 98.1 0.7	0.0 98.1 0.0	0.0 98.1 0.0
Male Feces+	% HPLC % in feces % dose total % dose	 2.5	 33.4	 0.9	 2.3	 6.7	27.0 4.5 1.2 45.1	16.6 4.5 0.7 8.6	14.2 4.5 0.6 1.3	0.0 4.5 0.0 0.0	15.9 4.5 0.7 0.7
Female Urine	% HPLC % in urine % dose	3.7 95.8 3.5	28.3 95.8 27.1	0.0 95.8 0.0	2.5 95.8 2.4	NR* 95.8 1.0	49.3 95.5 47.2	12.7 95.8 12.2	1.8 95.8 1.7	1.8 95.8 1.7	0.0 95.8 0.0
Female Feces+	% HPLC % in feces % dose total % dose	 3.5	 27.1	 0.0	 2.4	 1.0	28.2 5.1 1.4 48.6	13.9 5.1 0.7 12.9	14.3 5.1 0.7 2.4	0.0 5.1 0.0 1.7	19.1 5.1 1.0 1.0

a Extracted from Table V, page 41 of MRID 43830101
+ Only identified fecal metabolites included in tabulation.
NR* = U6 metabolite not resolved from U7.

Table 4:Metabolic Profiles of Whole Urine and Feces Extracts in Rats Treated with [^{14}C]2,4-DB [a]

Sex/Material	Material Balance	U1	2,4-D & 2,4-DCP Conjugates U2/U3	U4	2,4DB-OH Conjugate U5	2,4-DB Conjugate U6	2,4-D U7	2,4-DB-OH U8	2,4-DCP U9	U10	2,4-DB U11
Male Urine	% HPLC % in urine % dose	1.4 94.7 1.3	38.5 94.7 36.5	0.0 94.7 0.0	3.0 94.7 2.8	10.6 94.7 10.0	33.1 94.7 31.3	11.7 94.7 11.1	1.0 94.7 0.9	0.8 94.7 0.8	0.0 94.7 0.0
Male Feces+	% HPLC % in feces % dose total % dose	 1.3	 36.5	 0.0	 2.8	 10.0	38.7 6.7 2.6 33.9	3.9 6.7 0.3 11.4	13.7 6.7 0.9 1.8	0.0 6.7 0.0 0.8	13.8 6.7 0.9 0.9
Female Urine	% HPLC % in urine % dose	0.0 96.6 0.0	31.1 96.6 30.0	0.0 96.6 0.0	2.1 96.6 2.0	NR* 96.6 NR*	54.3 96.6 52.5	10.9 96.6 10.5	0.0 96.6 0.0	1.6 96.6 1.5	0.0 96.6 0.0
Female Feces+	% HPLC % in feces % dose total % dose	 0.0	 30.0	 0.0	 2.0	 NR*	26.7 4.3 1.1 53.6	15.0 4.3 0.6 11.1	14.1 4.3 0.6 0.6	0.0 4.3 0.0 1.5	26.0 4.3 1.1 1.1

a Extracted from Table VI page 42 of MRID 43830101
+ Only identified fecal metabolites included in tabulation.
NR* = U6 metabolite not resolved from U7.

Table 5: Quantitative Analysis of Urinary Components in Pre- and Post-Hydrolyzed Urine from Female Rats Treated with [^{14}C]2,4-DB DMA [a]

	Metabolites							
	2,4-D conjugate	2,4-DCP-conjugate	2,4-DB & 2,4-DB-OH conjugate	2,4-D	2,4-DB-OH	2,4-DCP		2,4-DB
Fraction	U2	U3	U5/U6	U7	U8	U9	U10	U11
Pre-hydrolysis	8.9	23.2	6.4	44.7	14.4	1.8	0.7	0.0
Post-hydrolysis	0.0	0.0	0.9	52.7	16.0	25.1	1.8	2.5
Net change*	-8.9	-23.2	-5.5	+8.0	+1.6	+23.3	+1.1	+2.5

a Extracted from Table VII (page 43) of MRID 43830101
* (-) indicates loss of metabolite after hydrolysis; (+) indicates gain of metabolite after hydrolysis

III. DISCUSSION

A. Investigator's Conclusions - The study author concluded that urine and feces excretion patterns were found to be virtually identical between rats administered either 2,4-DB DMA (salt) or 2,4-DB (free acid). In both treated groups, most of the administered radiocarbon (96-97%) was recovered in the urine within 24 hours of administration, indicating that both compounds are readily bioavailable and were rapidly excreted in the urine. Fecal excretion accounted for <6% of the dose. Levels of $^{14}CO_2$ accounted for ≤0.1% of the dose. Material balances between treated groups were comparable, accounting for 103% of the dose. HPLC and TLC chromatographic analyses of urine from the treated groups showed that the metabolic profiles were quantitatively and qualitatively similar. HPLC analysis of whole urine showed that ten metabolites resolved, five were present at significant (≥5% of the dose) concentrations. The major urinary metabolite was identified as 2,4-dichlorophenoxy)acetic acid (2,4-D), accounting for 31-44% of the dose. Three other major urinary metabolites (U2, U3 and U6) were identified as conjugated forms of 2,4-D, 2,4-dichlorophenol (2,4-DCP) and 2,4-DB, respectively. HPLC analysis of urine from female rats treated with 2,4-DB DMA showed that metabolites U2, U3 and U5/U6 accounted for 9, 23 and 6% of the urinary radiocarbon, respectively. An unknown urinary metabolite (U8), which accounted for 8-12% of the dose, was identified by mass spectrometry as 4-(2,4-dichlorophenoxy)-3-hydroxybutyric acid (2,4-DB-OH).

HPLC analysis of fecal extracts also showed that the metabolic profiles were quantitatively and qualitatively similar. Metabolites identified in the feces were 2,4-D, 2,4-DB-OH and 2,4-DCP, accounting for 1.1-2.5, 0.3-0.8 and 0.6-0.9% of the dose, respectively. Trace amounts of unmetabolized 2,4-DB accounted for 0.8-1.1% of the dose.

Based on the similarities in absorption, distribution , excretion and metabolism of [^{14}C]2,4-DB DMA and [^{14}C]2,4-DB, it was concluded that the salt dissociates to the free acid in the rat stomach and the two chemicals can be considered equivalent *in vivo*. Both the salt and the acid share the same metabolic pathway in the rat, which includes oxidation, hydrolysis and conjugation.

It is noted that some of the percentages stated in the conclusions do not agree with the data in Tables V and VI of the study report.

B. Reviewer's Discussion -

In this rat metabolism study (MRID 43830101), two adult CD (Crl:CD® (SB)BR) rats/sex/group were administered a single oral (gavage) dose of [^{14}C]2,4-DB acid or [^{14}C]2,4-DB DMA at target doses of 5.0 mg/kg or 6.0 mg/kg, respectively. Actual doses were

5.3 and 6.7 mg/kg. Three rats/sex/group served as a vehicle control (2% aqueous methylcellulose).

With both treated groups, the majority of the radioactivity (96-97% of the dose) was excreted in the urine. Fecal excretion accounted for ≤6% of the dose. Levels of expired $^{14}CO_2$ accounted for ≤0.1% of the dose. Material balances between treated groups were comparable, accounting for 102-104% of the dose.

HPLC analysis of whole urine showed that ten metabolites were chromatographically resolved. Of these, five metabolites accounted for ≥5% of the dose. The major urinary metabolite was identified as 2,4-dichlorophenoxy)acetic acid (2,4-D), accounting for 31-44% and 47-53% of the dose in males and females, respectively. Three other major urinary metabolites (U2, U3 and U6) were identified as conjugated forms of 2,4-D, 2,4-dichlorophenol (2,4-DCP) and 2,4-DB, respectively. HPLC analysis of urine from female rats treated with 2,4-DB DMA showed that metabolites U2, U3 and U5/U6 accounted for 9, 23 and 6% of the urinary radiocarbon, respectively. An unknown urinary metabolite (U8), which accounted for 8-12% of the dose, was identified by mass spectrometry as 4-(2,4-dichlorophenoxy)-3-hydroxybutyric acid (2,4-DB-OH).

HPLC analysis of fecal extracts identified metabolites as 2,4-D, 2,4-DB-OH and 2,4-DCP, accounting for 1.1-2.6, 0.3-0.7 and 0.6-0.9% of the dose, respectively. Trace amounts of unmetabolized 2,4-DB accounted for 0.7-1.1% of the dose.

Based on the analyses, a metabolic pathway was proposed. It appears that 2,4-DB DMA dissociates to the 2,4-DB (free acid) and then shares the same metabolic pathway as the 2,4-DB acid, which includes oxidation, hydrolysis and conjugation.

This metabolism study in the rat is classified acceptable (non-guideline) and does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rat. The study was originally intended as a pilot metabolism study and as such, does not meet the guideline requirements.

C. Study deficiencies -

1) The metabolism study guidelines require a minimum of 4 males for the Tier 1 testing, whereas this study had two males and two females per group.

2) The metabolism of the 2,4-DB acid and salt were compared via only one route of exposure (oral).

3) The metabolism guidelines require the determination of radioactivity in the urine at 6, 12 and 24 hours on day 1, whereas collection in this study was at 0.3, 1 and 2 days post-dosing.